Supplemental Material

Improved Reagents for Newborn Screening of Mucopolysaccharidosis-I, II and VI by Tandem Mass Spectrometry

Naveen Chennamaneni[1,4], Arun Babu Kumar[1,4], Mariana Barcenas[1], Zdeněk Spáčil [1], C. Ronald Scott[2], František Tureček [1], and Michael H. Gelb[*,1,3]

Departments of Chemistry, Pediatrics, and Biochemistry, University of Washington, Seattle, Washington 98195-1700

[1]Department of Chemistry

[2]Department of Pediatrics

[3]Department of Biochemistry

[4]These authors contributed equally to this study.

Table of Contents:

1. Synthesis of compounds

General information: All reactions were performed under ambient atmosphere unless specified otherwise. Flash column chromatography was performed usisng silica gel 60 Ang. (40-60 micron). NMR experiments were done using a 300 MHz Bruker instrument. The solvent peak was used as the internal standard with chemical shifts reported in ppm. Coupling constants are reported in Hz. Semi-preparative HPLC was carried out with a YMC-pack ODS-A (5 micron) 100x20 mm reverse phase column (Waters Inc.) with methano/water gradients.

***N*-(5-aminopentyl)benzamide (1a).** To methyl benzoate (1.0 g, 7.34 mmol), pentane-1,5-diamine (0.75 g, 7.34 mmol) and water (0.37 mL) were added, and the mixture was heated to 100°C for 24 hours under constant stirring. The reaction mixture was cooled to room temperature and directly loaded on to a short silica column (the silica column was pre-flushed with 4% triethylamine in chloroform followed by 100% chloroform before loading the reaction mixture). Upon elution with 30% of methanol in chloroform the desired mono-benzoylated product **1a** was obtained (0.80 g, 53%) as a pale yellow oil. ^{1}H NMR (300 MHz, MeOD) δ 7.83 (d, *J* = 7.4 Hz, 2H), 7.58 – 7.31 (m, 4H), 3.41 (t, *J* = 7.0 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 1.74 – 1.21 (m, 6H). MS *m/z* 207.2 $(M+H)^+$.

N-(6-aminohexyl)benzamide (1b). To methyl benzoate (25.0 g, 183.6 mmol), hexane-1,6-diamine (21.3 g, 183.6 mmol) and water (9.25 mL) were added and the mixture was heated to 100°C for 24 hours under constant stirring. The reaction mixture was cooled to room temperature and directly loaded on to a short silica column. Upon elution with 10 to 20% of methanol (with 5% NH_4OH) in chloroform the desired mono-benzoylated product **1b** was obtained (19.8 g, 49%) as a pale yellow oil. ^{1}H NMR (300 MHz, MeOD) δ 7.81 – 7.78 (m, 2H), 7.52 – 7.40 (m, 3H), 3.38 (t, *J* = 7.1 Hz, 2H), 2.78 – 2.69 (m, 2H), 1.64 – 1.38 (m, 8H). MS *m/z* 221.1 $(M+H^+)$.

N-(4-hydroxyphenyl)acrylamide (2). A solution of 4-aminophenol (50 g, 458 mmol) in CH_2Cl_2 (400 mL) and saturated $NaHCO_3$ in water (400 mL) was stirred for 10 min at room temperature, then acryloyl chloride (40.9 mL, 503.8 mmole) was added dropwise, and the reaction stirred for an additional 6 hr at room temperature. The resulting solid was collected by filtration, washed with water and dried under vacuum (oil pump) to afford 75 g of 4-acrylamido-phenol **2**. ^{1}H NMR (300 MHz, MeOD) δ 7.43 (dd, *J* = 6.8, 2.2 Hz, 2H), 6.77 (dd, *J* = 6.8, 2.2 Hz, 2H), 6.48 – 6.27 (m, 2H), 5.74 (dd, *J* = 9.5, 2.5 Hz, 1H).

***N*-(5-(*N*-(3-((4-hydroxyphenyl)amino)-3-oxopropyl)pentanamido)pentyl)benzamide (3a).** A solution of **1a** (207 mg, 1.00 mmol) and N-(4-hydroxyphenyl)acrylamide **2** (197 mg, 1.21 mmol) in *i*-propyl alcohol (9.0 mL) and water (1.0 mL) was heated to 65°C under constant stirring for 24 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure and further under high vacuum. To this crude concentrate anhydrous *N*,*N*-dimethylformamide (DMF) (3.0 mL) and triethylamine (253 mg, 2.50 mmol) were added, and the solids were allowed to completely dissolved. This solution was cooled to 0°C and valeryl chloride (241 mg, 2.00 mmol) was added dropwise and warmed to room temperature and stirred for 2 hours. The reaction was quenched with the addition of saturated sodium bicarbonate solution, and the reaction mixture was extracted with DCM/methanol (4:1). The organic layer was further washed with water and dried with anhydrous sodium sulfate. The organic layer was concentrated to dryness under reduced pressure and methanol (3.0 mL) was added to re-dissolve the residue. To this solution 5% aqueous sodium hydroxide (3.0 mL) was added dropwise and stirred at room temperature for 2 hours. The reaction was acidified, as indicated by pH paper, with 1N HCl solution and extracted with DCM/methanol (4:1). The organic layer

was concentrated under reduced pressure, and the residue was subjected to silica column chromatography and eluted with 5% methanol in DCM to yield **3a** (203 mg, 45%). ^{1}H NMR (300 MHz, MeOD) δ 8.45 (s, 1H), 7.83 – 7.78 (m, 2H), 7.56 – 7.40 (m, 3H), 7.34 – 7.26 (m, 2H), 6.76 – 6.68 (m, 2H), 3.69 (dt, *J* = 19.4, 6.9 Hz, 2H), 3.44 – 3.37 (m, 4H), 2.60 (dd, *J* = 14.7, 7.4 Hz, 2H), 2.47 – 2.30 (m, 2H), 1.75 – 1.49 (m, 6H), 1.47 – 1.26 (m, 4H), 0.91 (t, *J* = 7.3 Hz, 3H). MS *m/z* 476.5 $[M + Na]^+$.

N-(6-(N-(3-(4-hydroxyphenylamino)-3-oxopropyl)acetamido)hexyl)benzamide (3b). 4-Acrylamido-phenol **2** (8.43 g, 51.6 mmol) and mono-benzoyl-1,6-hexanediamine **1b** (12.5 g, 56.8 mmol) were dissolved in a solution of isopropanol (450 mL) and water (50 mL) and heated in an oil bath at 65^{o}C for 48 hrs. The reaction mixture was concentrated by rotary evaporation to afford the Michael addition product, which was divided into 2 parts and used for the next step without further purification.

To the residue from the above step was added CH_2Cl_2 (100 mL), DMF (10 mL) and 100 mL of saturated sodium bicarbonate in water. Acetyl chloride (3.7 mL, 52 mmol) was added dropwise at room temperature with stirring, and the mixture was stirred for an additional 6 h at room temperature. The organic layer was separated, and the water layer was extracted twice with 50 mL portions of 5% MeOH in CH_2Cl_2. The organic layers were combined and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (1-5% MeOH in CH_2Cl_2) to afford MPS-I aglycone **3b** (4.5 g, 10.6 mmol) in 41 % yield. ^{1}H NMR (300 MHz, MeOD) δ 7.88 – 7.76 (m, 2H), 7.58 – 7.39 (m, 3H), 7.36 – 7.24 (m, 2H), 6.78 – 6.69 (m, 2H), 3.70 (dt, *J* = 19.1, 7.0 Hz, 2H), 3.43 – 3.26 (m, 4H), 2.62 (dt, *J* = 10.2, 6.9 Hz, 2H), 2.13 (d, *J* = 16.4 Hz, 2H), 1.67-1.39 (m, 8H). MS *m/z* 426.5 ($M+H^+$).

N-(6-(N-(3-(4-hydroxyphenylamino)-3-oxopropyl)pentanamido)hexyl)benzamide (3c). To the Michael addition product from the above step was added CH_2Cl_2 (100 mL), DMF (10 mL) and 100 mL of saturated sodium bicarbonate in water. Pentanoyl chloride (6.17 mL, 52 mmol) was added dropwise at room temperature with stirring, and the mixture was stirred for an additional 6 h at room temperature. The organic layer was separated, and the water layer was extracted twice with 50 mL portions of 5% MeOH in CH_2Cl_2. The organic layers were combined and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (1-5% MeOH in CH_2Cl_2) to afford the MPS –II aglycone **3c** (3.5 g, 7.5 mmol) in 29 % yield. ^{1}H NMR (300 MHz, MeOD) δ 7.92 – 7.75 (m, 2H), 7.53-7.44 (m, 3H), 7.32 (d, *J* = 8.6 Hz, 2H), 6.83 – 6.67 (m, 2H), 3.70 (dt, *J* = 19.9, 6.7 Hz, 2H), 3.49 – 3.28 (m, 4H), 2.64-2.59 (m, 2H), 2.52 – 2.27 (m, 2H), 1.76 – 1.30 (m, 12H), 0.93 (td, *J* = 7.3, 4.5 Hz, 3H). MS *m/z* 468.5 ($M+H^+$).

(2S,3R,4S,5S,6R)-2-(4-(3-(N-(6-benzamidohexyl)acetamido)propanamido)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5b). MPS-I aglycone **3b** (2.5 g, 5.87 mmol, 1eq), methyl 2,3,4-triacetoxy-iduronosy-1-F **4** (2.37 g, 7.0 mmol, 1.2 eq) {Blanchard, 2008 #4618} and 2,6-di-*tert*-butyl-4-methylpyridine (3.62 g, 17.62 mmol, 3 eq) were dried for 1 hr under high vacuum (oil pump) and dissolved in dry CH_2Cl_2 (290 mL, 0.02 M). All of the MPS-I aglycone was not dissolved before addition of BF_3-etherate. $BF_3.Et_2O$ (7.41 mL, 58.75 mmol, 10 eq) was added dropwise with stirring at room temperature under a nitrogen atmosphere. After the reaction mixture had been stirred for 2.5 h at room temperature, 150 mL of saturated aqueous $NaHCO_3$ was added. The aqueous layer was extracted with CH_2Cl_2 and the organic extracts were combined and washed with water, brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (CH_2Cl_2, then 1-4% MeOH in CH_2Cl_2) to afford product **5b** (1.74 g, 2.35 mmol) in 40% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 – 7.73 (m, 2H), 7.52 – 7.39 (m, 5H), 6.99 (d, *J* = 8.9 Hz, 2H), 5.69 (s, 1H), 5.19-5.16 (m, 2H), 5.02 (s, 1H), 4.95

(s, 1H), 3.77 – 3.61 (m, 2H), 3.48 (s, 3H), 3.45 – 3.23 (m, 4H), 2.66 (m, 2H), 2.15 (s, 3H), 2.10 - 2.06 (m, 9H), 1.65- 1.30 (m, 8H). MS *m/z* 742.2 ($M+H^+$).

(2S,3R,4S,5S,6R)-2-(4-(3-(N-(6-benzamidohexyl)pentanamido)propanamido)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5c). MPS-II aglycone **3c** (1.9 g, 4.06 mmol, 1eq), methyl 2,3,4-triacetoxy-iduronosy-1-F **4** (1.23 g, 3.66 mmol, 0.9 eq) and 2,6-di-*tert*-butyl-4-methylpyridine (2.5 g, 12.2 mmol, 3 eq) were dried for 1 hr under high vacuum (oil pump) and dissolved in dry CH_2Cl_2 (80 mL, 0.05 M). All of the MPS-II aglycone dissolved before addition of BF_3-etherate. $BF_3.Et_2O$ (5.1 mL, 40.6 mmol, 10 eq) was added dropwise with stirring at room temperature under a nitrogen atmosphere. After the reaction mixture had been stirred for 2.5 h at room temperature, 150 mL of saturated aqueous $NaHCO_3$ was added. The aqueous layer was extracted with CH_2Cl_2 and the organic extracts were combined and washed with water, brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (CH_2Cl_2, then 1-4% MeOH in CH_2Cl_2) to afford product **5c** (1.87 g, 2.38 mmol) in 65 % yield. ^{1}H NMR (500 MHz, MeOD) δ 7.81 – 7.77 (m, 2H), 7.54 – 7.40 (m, 5H), 7.07 – 7.02 (m, 2H), 5.71 (d, *J* = 2.3 Hz, 1H), 5.18 (m, 2H), 5.06 – 5.02 (m, 1H), 4.99 (d, *J* = 3.1 Hz, 1H), 3.75 (s, 3H), 3.74 - 3.64 (m, 2H), 3.38 – 3.34 (m, 4H), 2.61 (m, 2H), 2.46 – 2.31 (m, 2H), 2.15 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.67 – 1.51 (m, 6H), 1.42 - 1.33 (m, 6H), 0.91 (t, *J* = 7.4 Hz, 3H). MS *m/z* 784.8 ($M+H^+$).

(2R,3S,4S,5R,6S)-methyl 6-(4-(3-(N-(6-benzamidohexyl)acetamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylate (6b).
To a solution of coupled product **5b** (1.74 g, 2.35 mmol, 1 eq) in 50 mL of dry methanol (Aldrich) was added 0.5 M sodium methoxide in methanol (1.88 mL, 0.94 mmol, 0.4 eq) dropwise at 0 °C under a nitrogen atmosphere with stirring. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was neutralized with AG 50W-X8 resin (H+) and filtered. The filtrate was concentrated by rotary evaporation. Column chromatography on silica gel (1-6% MeOH in CH_2Cl_2) afforded product **6b** (1.33 g, 2.16 mmol) in 92% yield. ^{1}H NMR (300 MHz, MeOD) δ 7.87 – 7.74 (m, 2H), 7.57 – 7.36 (m, 5H), 7.09 (d, *J* = 9.0 Hz, 2H), 5.59 (s, 1H), 4.79 – 4.75 (m, 2H), 3.93 (m, 2H), 3.78-3.65 (m, 6H), 3.38 – 3.36 (m, 4H), 2.66- 2.60 (m, 2H), 2.14 (d, *J* = 16.9 Hz, 3H), 1.63-1.42 (m, 8H). MS *m/z* 616.3 ($M+H^+$).

(2R,3S,4S,5R,6S)-methyl 6-(4-(3-(N-(6-benzamidohexyl)pentanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylate (6c).
To a solution of coupled product **5c** (2.7 g, 3.44 mmol, 1 eq) in 75 mL of dry methanol (Aldrich) was added 0.5 M sodium methoxide in methanol (2.75 mL, 1.38 mmol, 0.4 eq) dropwise at 0 °C under a nitrogen atmosphere with stirring. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was neutralized with AG 50W-X8 resin (H+) and filtered. The filtrate was concentrated by rotary evaporation. Column chromatography on silica gel (1-6% MeOH in CH_2Cl_2) afforded product **6c** (2.1 g, 3.19 mmol) in 91 % yield. ^{1}H NMR (500 MHz, MeOD) δ 7.80 (d, *J* = 7.7 Hz, 2H), 7.54 – 7.41 (m, 5H), 7.10 – 7.04 (m, 2H), 5.58 (d, *J* = 3.4 Hz, 1H), 4.77 (d, *J* = 3.3 Hz, 1H), 3.96 – 3.87 (m, 2H), 3.76 (s, 3H), 3.75 – 3.63 (m, 3H), 3.41 – 3.34 (m, 4H), 2.64 – 2.57 (m, 2H), 2.46 – 2.32 (m, 2H), 1.69 – 1.53 (m, 6H), 1.47 – 1.30 (m, 6H), 0.91 (t, *J* = 7.5 Hz, 3H). MS *m/z* 659.0 ($M+H^+$).

(2R,3S,4S,5R,6S)-6-(4-(3-(N-(6-benzamidohexyl)acetamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (MPS – I Substrate).
Deacylated compound **6b** (1.5 g, 2.44 mmol, 1 eq) was dissolved in 150 mL of water/methanol (1:1) at room temperature. An aqueous solution of sodium hydroxide 0.1 M was added in increments of 0.1 eq of NaOH until the pH of the solution reached approximately 8 (pH paper). The pH was maintained by incremental additions of the 0.1 M NaOH solution as the reaction proceeded (~2 eq NaOH added). The reaction mixture was stirred overnight. The reaction mixture was neutralized with 1 M HCl and concentrated by rotary evaporation. The residue was purified by column chromatography on silica (5% MeOH and 1% AcOH in CH_2Cl_2, then 10% MeOH and 2% AcOH in CH_2Cl_2) to give product **MPS-I Substrate** (1.45 g, 2.41 mmol) in 98% yield. ^{1}H NMR (300 MHz, MeOD) δ 7.80 (d, *J* = 7.3 Hz, 2H), 7.56 – 7.40 (m, 5H), 7.11 (d, *J* = 8.7 Hz, 2H), 5.55 (s, 1H), 4.72 (s, 1H), 3.97- 3.92 (m, 2H), 3.74- 3.64 (m, 3H), 3.39 – 3.33 (m, 4H), 2.11 (d, *J* = 17.3 Hz, 3H), 1.64 – 1.38 (m, 8H). MS *m/z* 602.4 ($M+H^+$).

sodium (2R,3S,4S,5R,6S)-6-(4-(3-(N-(6-benzamidohexyl)pentanamido)propanamido)phenoxy)-3,4-dihydroxy-5-(sulfonatooxy)tetrahydro-2H-pyran-2-carboxylate (MPS–II Substrate).
Deacetylated compound **6c** (2 g, 3.04 mmol, 1 eq) was solubilized in anhydrous MeOH (120 mL) and dibutyltin(IV) oxide (1.13g, 4.56 mmol, 1.5 eq) was added. The reaction mixture was heated under reflux for 1 hour under nitrogen, after which time the dibutyltin oxide was completely dissolved. The reaction mixture was allowed to cool and was concentrated under vacuum. The residue was co-evaporated once with anhydrous toluene (100 mL) to remove traces of water. The residue was solubilized in anhydrous N,N-dimethylformamide (120 mL). Sulfur trioxide–trimethylamine complex (633.8 mg, 4.56 mmol, 1.5 eq) was added, and the reaction mixture was stirred at room temperature under nitrogen atmosphere for 24 h. The reaction mixture was quenched with MeOH (20 mL). The mixture was then concentrated under vacuum. The residue was purified by column chromatography on silica gel (10% MeOH and 1% H_2O in CH_2Cl_2, then 20% MeOH and 2% H_2O in CH_2Cl_2) to give sulfate compound (1.2 g, 1.63 mmol) in 53.6% yield. ^{1}H NMR (300 MHz, MeOD) δ 7.81 (d, *J* = 8.4 Hz, 2H), 7.57 – 7.34 (m, 5H), 7.09 (d, *J* = 9.0 Hz, 2H), 5.83 (s, 1H), 4.48 (s, 1H), 4.16 (d, *J* = 2.7 Hz, 1H), 3.97 (s, 1H), 3.78 – 3.55 (m, 6H), 3.46 – 3.33 (m, 4H), 2.68 – 2.55 (m, 2H), 2.47 – 2.27 (m, 2H), 1.71 – 1.47 (m, 6H), 1.46 – 1.24 (m, 6H), 0.91 (t, *J* = 7.2 Hz, 3H). MS *m/z* 736.6 $(M-H)^-$

Sulfate compound (1 g, 1.35 mmol) was solubilized in 1:1 methanol–water (100 mL) at room temperature. An aqueous solution of 0.1 M NaOH was added in increments of 0.1 equiv of NaOH until the pH of the solution reached approximately 8 (pH paper). The pH was maintained by incremental additions of the 0.1 M NaOH solution as the reaction proceeded (every 15–30 min). It is probably important not to go to high in pH as this may result in some hydrolysis of the sulfate ester. The reaction mixture was stirred for overnight (~ 2 eq NaOH added), after which it was concentrated under vacuum to remove methanol and water. The residue was purified by column chromatography on silica gel (10% MeOH and 1% H_2O in CH_2Cl_2, then 20% MeOH and 2% H_2O in CH_2Cl_2) to give **MPS–II Substrate** (0.77 g, 1.06 mmol) in 79% yield. ^{1}H NMR (300 MHz, MeOD) δ 7.91 – 7.79 (m, 2H), 7.59 – 7.41 (m, 5H), 7.16 (d, *J* = 7.7 Hz, 2H), 5.79 (s, 1H), 4.66 (d, *J* = 3.3 Hz, 1H), 4.46 - 4.45 (m, 1H), 4.12 (m, 1H), 3.99 – 3.95 (m, 1H), 3.79 – 3.59 (m, 2H), 3.45 – 3.34 (m, 4H), 2.70 – 2.54 (m, 2H), 2.47 – 2.28 (m, 2H), 1.71 – 1.49 (m, 6H), 1.46 – 1.24 (m, 6H), 0.91 (t, *J* = 7.3 Hz, 3H). MS *m/z* 722.7 $(M-H)^-$

***N*-(5-(*N*-(3-((4-(((2S,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)amino)-3-oxopropyl)pentanamido)pentyl)benzamide (MPS-VI Product).**

To a solution of **3a** (148 mg, 0.326 mmol) and **7** (239 mg, 0.653 mmol) in DCM (0.4 mL) tetrabutylammonium hydrogen sulfate (110 mg, 0.324 mmol) and 2 M aqueous sodium hydroxide solution (0.4 mL) were added and left to stir for 3 hours at room temperature. To the reaction mixture another portion of **7** (90 mg, 0.246 mmol) was added and stirred for another 13 hours. The reaction mixture was then extracted between water and DCM and the organic layer was further washed with water, dried with anhydrous sodium sulfate and concentrated under reduced pressure. The resultant crude was purified by flash silica column chromatography using 4% methanol in DCM as eluent to get the peracetylated intermediate. The NMR spectroscopy indicated that the peracetylated intermediate has co-eluted with the starting material **3a**. This mixture was used for the next deacetylation step without further purification. To the solution of the above mixture in anhydrous methanol (5.0 mL), a 0.5 M solution of sodium methoxide in methanol (200 µL) was added dropwise at 0°C and left to stir for 2 hours at room temperature. The reaction was quenched with the addition of formic acid (100 µL) and subjected to semi-preparative reverse phase HPLC purification (gradient water/methanol system) to get **MPS-VI Product** (29 mg, 14%). ^{1}H NMR (300 MHz, MeOD) δ 7.83 (d, *J* = 7.1 Hz, 2H), 7.59 – 7.35 (m, 5H), 7.06 – 6.90 (m, 2H), 4.99 (d, *J* = 8.4 Hz, 1H), 4.27 – 4.11 (m, 1H), 3.96 – 3.55 (m, 7H), 3.46 – 3.35 (m, 4H), 2.71 – 2.51 (m, 2H), 2.49 – 2.27 (m, 2H), 2.01 (s, 3H), 1.76 – 1.49 (m, 6H), 1.37 (dd, *J* = 14.6, 7.2 Hz, 4H), 0.93 (t, *J* = 7.2 Hz, 3H). MS *m/z* 679.7 $[M + Na]^+$.

sodium (2R,3R,4R,5R,6S)-5-acetamido-6-(4-(3-(*N*-(5-benzamidopentyl)pentanamido)propanamido)phenoxy)-4-hydroxy-2-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl sulfate (MPS-VI Substrate).

To a cooled (0°C) solution of **MPS-VI Product** (25 mg, 38.1 µmol) in anhydrous pyridine (0.5 mL), benzoyl chloride (4.9 µL, 41.9 µmol) was added. After 1 hour at room temperature the solution was cooled back to 0°C and another portion of benzoyl chloride (9.4 µL, 80.4 µmol) was added and left to stir for 2 hours at room temperature. The reaction was extracted between 1 M HCl solution and chloroform. The chloroform layer was further washed with a mixture of water and brine solution (1:1). The organic layer was concentrated and purified by flash silica column chromatography using 5% methanol in DCM as the eluent. The desired fractions were concentrated under reduced pressure and further under high vacuum. The resultant residue was dissolved in anhydrous pyridine and sulfur trioxide pyridine complex (8.3 mg, 52.1 µmol) was added to it at room temperature. The resulting mixture was heated to 45°C for 3 hours followed by the addition of methanol (0.5 mL) and stirred for further 10 mins. The reaction mixture was concentrated under reduced pressure and further under high vacuum. The resulting residue was re-dissolved in anhydrous methanol (5.0 mL) and cooled to 0°C. To this cooled solution 0.5 M solution of sodium methoxide in methanol (0.5 mL) was added dropwise and let stir for 16 hours. The reaction was quenched by the addition of 1 M aqueous solution of sodium phosphate monobasic (1.0 mL) and subjected to semi-preparative reverse phase HPLC purification (gradient water/methanol system) to yield **MPS-VI Substrate** (5.8 mg, 20%). ^{1}H NMR (300 MHz, MeOD) δ 7.80 (dd, *J* = 7.0, 1.2 Hz, 2H), 7.58 – 7.38 (m, 5H), 7.03 – 6.94 (m, 2H), 5.01 (dd, *J* = 8.4, 1.1 Hz, 1H), 4.75 (d, *J* = 3.1 Hz, 1H), 4.13 (dd, *J* = 10.9, 8.4 Hz, 1H), 3.95 – 3.61 (m, 6H), 3.45 – 3.34 (m, 4H), 2.61 (dd, *J* = 16.1, 7.0 Hz, 2H), 2.47 – 2.31 (m, 2H), 1.97 (s, 3H), 1.73 – 1.49 (m, 6H), 1.43 – 1.23 (m, 5H), 0.91 (td, *J* = 7.3, 2.2 Hz, 3H). MS *m/z* 735.4 $[M - Na^+]^-$.

N-(6-(N-(3-(4-hydroxyphenylamino)-3-oxopropyl)acetamido)hexyl)-d_5-benzamide (MPS-I Internal Standard).

4-Acrylamido-phenol **2** (142 mg, 0.87 mmol) and mono-BOC-1,6-hexanediamine (Ark Pharm Inc.) (207 mg, 0.96 mmol) were dissolved in a solution of isopropanol (9 mL) and water (1 mL) and heated in an oil bath at 65°C for 48 hrs. The reaction mixture was concentrated by rotary evaporation to afford the Michael addition product **8a**, which was used in the next step without further purification.
To the Michael addition product **8a** was added CH_2Cl_2 (3 mL) and 3 mL of saturated sodium bicarbonate in water. Acetyl chloride (0.18 mL, 2.63 mmole) was added dropwise at room temperature with stirring, and the mixture was stirred for an additional 3 h at room temperature. The layers were allowed to separate, and the CH_2Cl_2 layer was concentrated by rotary evaporation.

The residue was dissolved in 3 mL of CH_2Cl_2 and 1.5 mL of 4 M HCl in dioxane was added dropwise with stirring. Stirring was continued at room temperature for 1 hr. The resulting solid was collected by filtration, and the solid was dried under vacuum (oil pump).

To the above solid was added 5 mL of CH_2Cl_2 and 5 mL of saturated sodium bicarbonate in water. d_5-benzoyl chloride (0.11 mL, 0.87 mmole) was added dropwise with stirring, and the mixture was stirred an additional 3 hr at room temperature. The layers were allowed to separate, and the CH_2Cl_2 layer was concentrated with a rotary evaporator. The residue was dissolved in 2 mL of MeOH, and 2 mL of 5% NaOH in water was added. The mixture was stirred for 30 min at room temperature (this step is necessary to remove any benzoylated phenol). The mixture was neutralized with 1 M HCl and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and solvent was removed by rotary evaporation. The residue was submitted to silica gel chromatography with 5% MeOH in CH_2Cl_2 to give 110 mg of pure product **MPS-I Internal Standard** (30% overall yield). ^{1}H NMR (500 MHz, MeOD) δ 7.31 (d, *J* = 9.0 Hz, 2H), 6.79 – 6.63 (m, 2H), 3.68 (dt, *J* = 32.5, 6.9 Hz, 2H), 3.46 – 3.33 (m, 4H), 2.60 (dt, *J* = 17.5, 6.8 Hz, 2H), 2.12 (d, *J* = 27.0 Hz, 3H), 1.68-1.55 (m, 4H), 1.46-1.35 (m, 4H). MS *m/z* 431.6 ($M+H^+$).

N-(6-(N-(3-(4-hydroxyphenylamino)-3-oxopropyl)pentanamido)hexyl)-d_5-benzamide (9).
4-Acrylamido-phenol **2** (429 mg, 2.63 mmol) and mono-BOC-1,6-hexanediamine (Ark Pharm Inc.) (625 mg, 2.89 mmol) were dissolved in a solution of isopropanol (18 mL) and water (2 mL) and heated in an oil bath at 65°C for 48 hrs. The reaction mixture was concentrated by rotary evaporation to afford the Michael addition product **8b**, which was used in the next step without further purification.

To the Michael addition product **8b** was added CH_2Cl_2 (10 mL) and 10 mL of saturated sodium bicarbonate in water. Pentanoyl chloride (0.94 mL, 7.89 mmole) was added dropwise at room temperature with stirring, and the mixture was stirred for an additional 3 h at room temperature. The layers were allowed to separate, and the CH_2Cl_2 layer was concentrated by rotary evaporation.

The residue was dissolved in 10 mL of CH_2Cl_2 and 5 mL of 4 M HCl in dioxane was added dropwise with stirring. Stirring was continued at room temperature for 1 hr. The resulting solid was collected by filtration, and the solid was dried under vacuum (oil pump).

To the above solid was added 10 mL of CH_2Cl_2 and 10 mL of saturated sodium bicarbonate in water. d_5-benzoyl chloride (0.34 mL, 2.89 mmole) was added dropwise with stirring, and the mixture was stirred an additional 3 hr at room temperature. The layers were allowed to separate, and the CH_2Cl_2 layer was concentrated with a rotary evaporator. The residue was dissolved in 4 mL of MeOH, and 4 mL of 5% NaOH in water was added. The mixture was

stirred for 30 min at room temperature (this step is necessary to remove any benzoylated phenol). The mixture was neutralized with 1 M HCl and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and solvent was removed by rotary evaporation. The residue was submitted to silica gel chromatography with 5% MeOH in CH_2Cl_2 to give 570 mg of pure product **9.** ^{1}H NMR (500 MHz, MeOD) δ 7.31 (d, *J* = 8.9 Hz, 2H), 6.73 (dd, *J* = 9.0, 2.9 Hz, 2H), 3.68 (dt, *J* = 33.4, 7.0 Hz, 2H), 3.44-3.35 (m, 4H), 2.67 – 2.52 (m, 2H), 2.51 – 2.28 (m, 2H), 1.72 – 1.30 (m, 12H), 1.00 – 0.83 (m, 3H). MS *m/z* 473.6 ($M+H^+$).

(2S,3R,4S,5S,6R)-2-(4-(3-(N-(6-d_5-benzamidohexyl)pentanamido)propanamido)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (10).
MPS-II aglycone **9** (0.57g, 1.2 mmol, 1eq), methyl 2,3,4-triacetoxy-iduronosy-1-F **4** (0.36g, 1.08 mmol, 0.9 eq) and 2,6-di-*tert*-butyl-4-methylpyridine (0.74 g, 3.6 mmol, 3 eq) were dried for 1 hr under high vacuum (oil pump) and dissolved in dry CH_2Cl_2 (24 mL, 0.05 M). $BF_3.Et_2O$ (1.51 mL, 12 mmol, 10 eq) was added dropwise with stirring at room temperature under a nitrogen atmosphere. After the reaction mixture had been stirred for 2.5 h at room temperature, 100 mL of saturated aqueous $NaHCO_3$ was added. The aqueous layer was extracted with CH_2Cl_2 and the organic extracts were combined and washed with water, brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (CH_2Cl_2, then 1-4% MeOH in CH_2Cl_2) to afford product **10** (0.69 g, 0.87 mmol) in 72% yield. ^{1}H NMR (500 MHz, MeOD) δ 7.48 (dt, *J* = 9.2, 2.5 Hz, 2H), 7.09 – 6.97 (m, 2H), 5.71 (d, *J* = 2.5 Hz, 1H), 5.18 (m, 2H), 5.06 – 5.01 (m, 1H), 4.99 (d, *J* = 3.1 Hz, 1H), 3.74 – 3.62 (m, 5H), 3.40 – 3.33 (m, 4H), 2.62 (dt, *J* = 13.6, 6.9 Hz, 2H), 2.47 – 2.31 (m, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.65-1.30 (m, 12H), 0.97 – 0.84 (m, 3H). MS *m/z* 789.8 ($M+H^+$).

(2R,3S,4S,5R,6S)-6-(4-(3-(N-(6-d_5-benzamidohexyl)pentanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (MPS–II Internal Standard).
To a solution of coupled product **10** (0.69 g, 0.87 mmol, 1 eq) in 18 mL of dry methanol (Aldrich) was added 0.5 M sodium methoxide in methanol (0.69 mL, 0.35 mmol, 0.4 eq) dropwise at 0 °C under a nitrogen atmosphere with stirring. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was neutralized with AG 50W-X8 resin (H+) and filtered. The filtrate was concentrated by rotary evaporation. Column chromatography on silica gel (1-6% MeOH in CH_2Cl_2) afforded product (0.41 g, 0.62 mmol) in 72 % yield. ^{1}H NMR (300 MHz, MeOD) δ 7.45 (d, *J* = 8.9 Hz, 2H), 7.14 – 6.94 (m, 2H), 5.58 (d, *J* = 3.5 Hz, 1H), 4.78 (d, *J* = 3.0 Hz, 1H), 3.93 – 3.90 (m, 2H), 3.75 (s, 3H), 3.73 – 3.61 (m, 4H), 3.41 – 3.27 (m, 4H), 2.62 – 2.58 (m, 2H), 2.47 – 2.26 (m, 2H), 1.70 – 1.24 (m, 12H), 0.93 – 0.86 (m, 3H).

Deacylated compound (0.41 g, 0.61 mmol, 1 eq) was dissolved in 40 mL of water/methanol (1:1) at room temperature. An aqueous solution of sodium hydroxide 0.1 M was added in increments of 0.1 eq of NaOH until the pH of the solution reached approximately 8 (pH paper). The pH was maintained by incremental additions of the 0.1 M NaOH solution as the reaction proceeded (~2 eq NaOH added). The reaction mixture was stirred overnight. The reaction mixture was neutralized with 1 M HCl and concentrated by rotary evaporation. The residue was purified by column chromatography on silica (5% MeOH and 1% AcOH in CH_2Cl_2, then 10% MeOH and 2% AcOH in CH_2Cl_2) to give product **MPS-II Internal Standard** (0.39 g, 0.6 mmol) in 96% yield. ^{1}H NMR (300 MHz, MeOD) δ 7.44 (dd, *J* = 9.1, 1.5 Hz, 2H), 7.24 (dd, *J* = 9.1, 3.2 Hz, 2H), 5.51 (d, *J* = 4.9 Hz, 1H), 4.53 (d, *J* = 3.9 Hz, 1H), 3.88 – 3.53 (m, 6H), 3.41 – 3.32 (m, 4H), 2.61-2.56 (m, 2H), 2.46 – 2.25 (m, 2H), 1.71 – 1.21 (m, 12H), 0.94 – 0.86 (m, 3H). MS *m/z* 649.6 ($M+H^+$).

2. Tandem mass spectrometry instrument settings.

Waters XEVO TQ experimental parameters for flow-injection tandem mass spectrometry assay.

Parameter (units)	
Capillary voltage (V)	3000
Extractor (V)	3.00
Source temperature (°C)	90
Desolvation temperature (°C)	150
Cone Gas Flow (L/h)	50
Desolvation Gas Flow (L/h)	450
LM 1 Resolution	2.6
HM 1 Resolution	15.0
Ion Energy	0.5
Collision Cell Entrance Potential (V)	0.50
Collision Cell Exit Potential (V)	0.50
LM 2 Resolution	2.8
HM 2 Resolution	14.7
Ion Energy 2	0.6
Multiplier (V)	493.04
Collision Gas	Argon

Analyte	**SRM transition (*m/z*)**	**Cone Voltage (V)**	**Collision Energy (eV)**
Original MPS-I Substrate	567.26 → 467.20	7	11
Original MPS-I Product	391.19 → 291.13	7	11
Original MPS-I Int. Std.	377.17 → 277.12	7	11
MPS-I Substrate	602.23→ 317.16	18	16
MPS- I Product	426.20→ 317.17	24	16
MPS-I Int. Std.	431.23→ 322.20	24	16

Original MPS-II Substrate	697.20 → 597.20	10	11
Original MPS-II Product	595.25 → 495.20	10	11
Original MPS-II Int. Std.	604.31 → 496.20	10	11
MPS-II Substrate	724.60→ 359.17	15	26
MPS-II Product	644.26→ 359.16	15	20
MPS-II Int. Std.	649.36 → 364.26	16	20
Original MPS-VI Substrate	724.24 → 624.24	12	11
Original MPS-VI Product	622.30 → 522.24	12	11
Original MPS-VI Int. Std.	608.28 → 508.23	12	11
MPS-VI Substrate	737.39→ 345.24	15	16
MPS-VI Product	657.45→ 345.19	18	20
MPS-VI Int. Std.	662.50→ 350.25	18	20

3. Flow-injection parameters

The flow-injection solvent is 80:20 (v/v) methanol:water with 5 mM ammonium formate. The flow stream from the autosampler during injection is 0.3 mL/min. After sample injection, the flow rate is dropped to 0.2 ml/min for 0.1 min, then dropped to 0.03 ml/min for 0.15 min, and held at 0.03 mL/min for 0.65 min, then raised to 0.4 mL/min at 0.9 min, then to 0.3 mL/min at 1 min. The idea is to flow the sample quickly into the ESI source and then to reduce the flow rate during the time in which mass spectrometry data is collected. We inject 10 uL per well. Total run time is 1.5 min.

4. Supplemental Table 1. Data for MPS-I

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
Blank	3.44E+03	7.92E+05	2.73E+05	0.004	0.013		
Blank	3.34E+03	7.98E+05	2.78E+05	0.004	0.012		
Blank	3.44E+03	8.34E+05	2.94E+05	0.004	0.012	0.012	2.678
Blank	3.64E+03	8.34E+05	3.51E+05	0.004	0.013		
Blank	3.59E+03	8.54E+05	3.52E+05	0.004	0.012		
Blank	3.68E+03	8.65E+05	3.66E+05	0.004	0.012	0.013	1.985

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
DBS_healthy_1	1.82E+06	7.25E+05	1.06E+05	2.511	7.358		
DBS_healthy_1	1.84E+06	7.51E+05	1.05E+05	2.447	7.169		
DBS_healthy_1	1.87E+06	7.44E+05	1.06E+05	2.519	7.380	7.302	1.586
DBS_healthy_2	6.42E+05	6.87E+05	8.18E+04	0.933	2.735		
DBS_healthy_2	6.49E+05	6.92E+05	8.23E+04	0.937	2.746		
DBS_healthy_2	6.63E+05	6.99E+05	7.96E+04	0.948	2.778	2.753	0.822
DBS_healthy_3	1.10E+06	8.31E+05	1.13E+05	1.330	3.896		
DBS_healthy_3	1.11E+06	8.46E+05	1.12E+05	1.314	3.849		
DBS_healthy_3	1.13E+06	8.53E+05	1.13E+05	1.328	3.891	3.879	0.669
DBS_healthy_4	5.90E+05	8.03E+05	9.69E+04	0.735	2.153		
DBS_healthy_4	5.98E+05	8.05E+05	9.65E+04	0.743	2.177		
DBS_healthy_4	5.98E+05	8.13E+05	9.52E+04	0.736	2.156	2.162	0.600
DBS_healthy_5	8.55E+05	7.83E+05	7.96E+04	1.092	3.199		

DBS_healthy_5	8.48E+05	7.79E+05	7.86E+04	1.088	3.188		
DBS_healthy_5	8.59E+05	7.90E+05	7.83E+04	1.087	3.184	3.190	0.236
DBS_healthy_6	6.29E+05	7.28E+05	6.35E+04	0.864	2.531		
DBS_healthy_6	6.17E+05	7.24E+05	6.38E+04	0.852	2.495		
DBS_healthy_6	6.20E+05	7.31E+05	6.41E+04	0.849	2.487	2.505	0.935
DBS_healthy_7	5.72E+05	5.18E+05	6.61E+04	1.104	3.235		
DBS_healthy_7	5.87E+05	5.22E+05	6.62E+04	1.123	3.291		
DBS_healthy_7	5.82E+05	5.25E+05	6.67E+04	1.109	3.249	3.258	0.887
DBS_healthy_8	6.76E+05	7.93E+05	7.70E+04	0.852	2.496		
DBS_healthy_8	6.72E+05	8.00E+05	7.62E+04	0.840	2.461		
DBS_healthy_8	6.83E+05	8.00E+05	7.71E+04	0.853	2.500	2.486	0.868
DBS_healthy_9	7.33E+05	6.22E+05	8.70E+04	1.177	3.450		
DBS_healthy_9	7.32E+05	6.20E+05	8.53E+04	1.181	3.460		
DBS_healthy_9	7.39E+05	6.28E+05	8.55E+04	1.177	3.447	3.453	0.202
DBS_healthy_10	5.61E+05	5.46E+05	5.58E+04	1.027	3.008		
DBS_healthy_10	5.67E+05	5.55E+05	5.83E+04	1.021	2.991		
DBS_healthy_10	5.74E+05	5.56E+05	5.65E+04	1.034	3.029	3.009	0.648
DBS_healthy_11	6.83E+05	7.12E+05	8.13E+04	0.959	2.811		
DBS_healthy_11	6.89E+05	7.11E+05	7.94E+04	0.969	2.839		
DBS_healthy_11	6.95E+05	7.20E+05	7.97E+04	0.965	2.828	2.826	0.506
DBS_healthy_12	6.60E+05	6.31E+05	1.14E+05	1.045	3.063		
DBS_healthy_12	6.61E+05	6.35E+05	1.17E+05	1.042	3.053		
DBS_healthy_12	6.62E+05	6.36E+05	1.15E+05	1.040	3.048	3.054	0.256
DBS_healthy_13	5.83E+05	7.03E+05	1.02E+05	0.830	2.430		
DBS_healthy_13	5.90E+05	7.08E+05	1.02E+05	0.832	2.439		
DBS_healthy_13	5.93E+05	7.12E+05	1.02E+05	0.833	2.441	2.437	0.236
DBS_healthy_14	6.12E+05	7.19E+05	8.94E+04	0.852	2.495		
DBS_healthy_14	6.27E+05	7.22E+05	8.82E+04	0.868	2.544		

DBS_healthy_14	6.08E+05	7.22E+05	9.24E+04	0.842	2.467	2.502	1.562
DBS_healthy_15	6.20E+05	6.69E+05	9.79E+04	0.926	2.713		
DBS_healthy_15	6.16E+05	6.72E+05	1.00E+05	0.917	2.686		
DBS_healthy_15	6.17E+05	6.72E+05	1.00E+05	0.918	2.690	2.696	0.553

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
Affected individual	1.93E+04	7.15E+05	8.38E+04	0.027	0.079		
Affected individual	1.95E+04	7.18E+05	8.54E+04	0.027	0.080		
Affected individual	1.93E+04	7.16E+05	8.33E+04	0.027	0.079	0.079	0.480

Supplemental Table 2. Data for MPS-II

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
Blank	4.24E+03	2.10E+05	2.24E+03	0.020	0.059		
Blank	3.71E+03	2.14E+05	2.22E+03	0.017	0.051		
Blank	4.08E+03	2.15E+05	2.45E+03	0.019	0.056	0.055	7.538
Blank	1.87E+03	2.23E+05	3.08E+03	0.008	0.024		
Blank	2.35E+03	2.16E+05	2.85E+03	0.011	0.032		
Blank	2.01E+03	2.20E+05	2.58E+03	0.009	0.027	0.028	13.555

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
DBS_healthy_1	1.69E+05	4.97E+04	4.51E+02	3.400	9.962		
DBS_healthy_1	1.69E+05	5.18E+04	5.46E+02	3.271	9.584		
DBS_healthy_1	1.71E+05	5.09E+04	4.96E+02	3.354	9.825	9.790	1.955
DBS_healthy_2	1.52E+05	4.95E+04	5.33E+02	3.064	8.977		
DBS_healthy_2	1.55E+05	5.21E+04	5.06E+02	2.968	8.694		
DBS_healthy_2	1.60E+05	4.96E+04	5.87E+02	3.226	9.450	9.040	4.225
DBS_healthy_3	1.52E+05	4.59E+04	6.79E+02	3.309	9.695		
DBS_healthy_3	1.51E+05	4.67E+04	7.01E+02	3.229	9.460		
DBS_healthy_3	1.53E+05	4.56E+04	6.76E+02	3.352	9.821	9.658	1.897
DBS_healthy_4	1.69E+05	5.18E+04	5.50E+02	3.258	9.544		
DBS_healthy_4	1.66E+05	5.67E+04	4.74E+02	2.931	8.587		
DBS_healthy_4	1.68E+05	5.50E+04	7.27E+02	3.063	8.973	9.034	5.330
DBS_healthy_5	1.56E+05	4.82E+04	5.03E+02	3.227	9.455		

DBS_healthy_5	1.55E+05	5.01E+04	7.78E+02	3.095	9.068		
DBS_healthy_5	1.57E+05	5.00E+04	6.38E+02	3.133	9.178	9.233	2.159
DBS_healthy_6	1.91E+05	4.93E+04	7.73E+02	3.879	11.365		
DBS_healthy_6	1.92E+05	4.99E+04	6.58E+02	3.846	11.268		
DBS_healthy_6	1.90E+05	5.08E+04	4.98E+02	3.736	10.945	11.193	1.966
DBS_healthy_7	1.57E+05	5.13E+04	4.57E+02	3.050	8.934		
DBS_healthy_7	1.56E+05	5.20E+04	7.00E+02	3.006	8.807		
DBS_healthy_7	1.56E+05	5.18E+04	4.10E+02	3.008	8.811	8.851	0.816
DBS_healthy_8	1.61E+05	5.59E+04	4.82E+02	2.880	8.436		
DBS_healthy_8	1.64E+05	5.56E+04	6.81E+02	2.958	8.667		
DBS_healthy_8	1.62E+05	5.52E+04	9.46E+02	2.931	8.586	8.563	1.369
DBS_healthy_9	1.18E+05	5.38E+04	6.88E+02	2.188	6.411		
DBS_healthy_9	1.17E+05	5.42E+04	7.96E+02	2.165	6.341		
DBS_healthy_9	1.20E+05	5.43E+04	7.32E+02	2.202	6.450	6.401	0.860
DBS_healthy_10	1.54E+05	4.65E+04	4.86E+02	3.304	9.681		
DBS_healthy_10	1.54E+05	4.68E+04	3.89E+02	3.286	9.628		
DBS_healthy_10	1.54E+05	4.63E+04	3.60E+02	3.333	9.764	9.691	0.708
DBS_healthy_11	1.71E+05	5.42E+04	4.46E+02	3.158	9.252		
DBS_healthy_11	1.72E+05	5.27E+04	5.58E+02	3.258	9.544		
DBS_healthy_11	1.64E+05	5.26E+04	4.80E+02	3.122	9.147	9.315	2.207
DBS_healthy_12	1.59E+05	5.50E+04	6.43E+02	2.899	8.493		
DBS_healthy_12	1.58E+05	5.73E+04	7.44E+02	2.763	8.095		
DBS_healthy_12	1.61E+05	5.39E+04	7.09E+02	2.980	8.730	8.439	3.804
DBS_healthy_13	1.50E+05	5.94E+04	6.38E+02	2.527	7.402		
DBS_healthy_13	1.55E+05	5.83E+04	6.13E+02	2.666	7.811		
DBS_healthy_13	1.47E+05	6.08E+04	4.57E+02	2.423	7.098	7.437	4.808
DBS_healthy_14	1.68E+05	6.12E+04	8.41E+02	2.741	8.032		
DBS_healthy_14	1.71E+05	5.97E+04	7.77E+02	2.871	8.411		

DBS_healthy_14	1.67E+05	5.81E+04	6.72E+02	2.873	8.417	8.287	2.665
DBS_healthy_15	1.25E+05	5.42E+04	5.25E+02	2.310	6.767		
DBS_healthy_15	1.30E+05	5.90E+04	6.64E+02	2.199	6.441		
DBS_healthy_15	1.25E+05	5.39E+04	6.82E+02	2.323	6.806	6.672	3.007

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
Affected individual	7.15E+03	7.25E+04	8.71E+02	0.099	0.289		
Affected individual	6.24E+03	7.38E+04	1.13E+03	0.084	0.247		
Affected individual	6.66E+03	7.13E+04	1.06E+03	0.093	0.274	0.270	7.772

Supplemental Table 3. Data for MPS-VI

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
Blank	2.43E+02	3.08E+03	1.52E+02	0.079	0.231		
Blank	2.63E+02	2.98E+03	1.48E+02	0.088	0.259		
Blank	2.36E+02	3.45E+03	1.43E+02	0.069	0.201	0.230	12.610
Blank	2.44E+02	3.34E+03	7.90E+00	0.073	0.214		
Blank	2.30E+02	3.38E+03	5.60E+00	0.068	0.199		
Blank	2.25E+02	3.48E+03	1.01E+01	0.065	0.189	0.201	6.095

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
DBS_healthy_1	1.38E+04	2.64E+03	1.66E+01	5.239	15.349		
DBS_healthy_1	1.30E+04	2.54E+03	1.38E+01	5.110	14.972		
DBS_healthy_1	1.34E+04	2.65E+03	4.70E+00	5.039	14.762	15.028	1.982
DBS_healthy_2	2.88E+04	3.22E+03	1.90E+00	8.964	26.262		
DBS_healthy_2	2.90E+04	3.08E+03	1.99E+01	9.426	27.614		
DBS_healthy_2	2.87E+04	3.13E+03	1.22E+01	9.158	26.830	26.902	2.524
DBS_healthy_3	9.24E+03	2.89E+03	3.30E+00	3.200	9.375		
DBS_healthy_3	9.69E+03	2.69E+03	2.80E+00	3.609	10.573		
DBS_healthy_3	9.43E+03	2.58E+03	1.04E+01	3.656	10.710	10.220	7.188
DBS_healthy_4	7.99E+03	1.97E+03	5.80E+00	4.065	11.908		
DBS_healthy_4	7.96E+03	2.16E+03	5.00E+00	3.691	10.813		
DBS_healthy_4	7.71E+03	2.08E+03	6.00E+00	3.701	10.842	11.188	5.579
DBS_healthy_5	6.96E+03	2.21E+03	5.50E+00	3.149	9.225		

DBS_healthy_5	7.15E+03	2.04E+03	8.70E+00	3.503	10.262		
DBS_healthy_5	7.29E+03	2.31E+03	1.47E+01	3.150	9.228	9.572	6.245
DBS_healthy_6	1.48E+04	1.45E+03	3.60E+00	10.188	29.849		
DBS_healthy_6	1.50E+04	1.42E+03	1.36E+01	10.565	30.953		
DBS_healthy_6	1.47E+04	1.38E+03	1.55E+01	10.636	31.159	30.654	2.299
DBS_healthy_7	3.24E+04	2.65E+03	4.10E+00	12.203	35.752		
DBS_healthy_7	3.23E+04	2.76E+03	1.11E+01	11.702	34.284		
DBS_healthy_7	3.32E+04	2.77E+03	1.87E+01	12.009	35.182	35.073	2.111
DBS_healthy_8	1.34E+04	1.81E+03	7.50E+00	7.415	21.724		
DBS_healthy_8	1.34E+04	2.03E+03	6.50E+00	6.590	19.308		
DBS_healthy_8	1.36E+04	1.80E+03	1.12E+01	7.532	22.068	21.033	7.152
DBS_healthy_9	3.24E+04	2.91E+03	1.76E+01	11.124	32.590		
DBS_healthy_9	3.32E+04	3.15E+03	1.71E+01	10.552	30.914		
DBS_healthy_9	3.27E+04	3.07E+03	7.80E+00	10.640	31.173	31.559	2.860
DBS_healthy_10	2.27E+04	1.88E+03	6.62E+01	12.043	35.281		
DBS_healthy_10	2.18E+04	1.85E+03	1.55E+01	11.763	34.461		
DBS_healthy_10	2.16E+04	1.82E+03	1.06E+01	11.820	34.628	34.790	1.246
DBS_healthy_11	2.20E+04	1.90E+03	1.18E+02	11.566	33.886		
DBS_healthy_11	2.19E+04	1.99E+03	1.15E+02	11.006	32.244		
DBS_healthy_11	2.20E+04	1.84E+03	1.24E+02	11.916	34.910	33.680	3.992
DBS_healthy_12	9.38E+03	1.98E+03	1.93E+02	4.748	13.910		
DBS_healthy_12	9.70E+03	2.15E+03	1.61E+02	4.516	13.231		
DBS_healthy_12	9.11E+03	1.86E+03	1.28E+02	4.894	14.338	13.827	4.038
DBS_healthy_13	8.92E+03	1.52E+03	1.41E+02	5.857	17.158		
DBS_healthy_13	8.66E+03	1.61E+03	1.44E+02	5.374	15.745		
DBS_healthy_13	8.61E+03	1.50E+03	1.03E+02	5.757	16.868	16.590	4.496
DBS_healthy_14	4.01E+04	2.10E+03	1.14E+02	19.144	56.086		
DBS_healthy_14	3.99E+04	1.99E+03	1.27E+02	20.019	58.650		

DBS_healthy_14	3.97E+04	2.02E+03	1.01E+02	19.696	57.703	57.480	2.255
DBS_healthy_15	5.73E+03	4.27E+02	9.29E+01	13.441	39.378		
DBS_healthy_15	5.62E+03	4.67E+02	2.19E+01	12.041	35.275		
DBS_healthy_15	5.45E+03	4.54E+02	2.67E+01	11.994	35.138	36.597	6.583

	P	IS	S	P/IS	Enzyme Activity (umol/L*h)	Average	Intraassay CV
Affected individual	2.53E+03	4.96E+03	1.43E+01	0.510	1.496		
Affected individual	2.62E+03	4.91E+03	1.69E+01	0.533	1.562		
Affected individual	2.91E+03	5.16E+03	1.57E+01	0.564	1.652	1.570	5.015